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Case Report

Chronic prostatitis in a dog infected with a smooth strain of *Brucella* spp.

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Abstract

Dogs may be occasionally infected by smooth strains of *Brucella* spp. The infection is usually associated with the ingestion of contaminated material from parturition or abortion, or other tissues from infected farm animals, particularly cattle and pigs. A 6-year-old, male mixed breed dog from a rural area was admitted at a veterinary clinic for clinical examination. The dog had bilateral perineal hernia with dysuria and dyschezia, as well as small firm testicles with scrotal adhesions. Serological tests, including buffered plate antigen, serum agglutination test, and 2-mercaptoethanol test, were positive for smooth *Brucella* spp. strains, whereas a rapid slide agglutination test was negative for *B. canis*. Blood and prostate tissue samples yielded no bacterial isolates. Histopathology demonstrated interstitial lymphoplasmacytic and histiocytic infiltration of the prostate gland, with fibrosis and occasional disruption of glandular architecture. Immunohistochemistry demonstrated abundant *Brucella* spp. antigens in the cytoplasm of macrophages. This report supports the notion that not only *B. canis*, but also smooth *Brucella* spp. must be considered in the differential diagnosis of prostatitis in dogs.

Key words: *Brucella*, canine, prostate, pathology.

Introduction

Brucellosis is a chronic zoonotic infection caused by Gram negative bacteria of the genus *Brucella*, which are facultative intracellular pathogens with tropism for the lymphoreticular and reproductive systems (9). Classical *Brucella* species affecting domestic animals include: *Brucella abortus*, *B. suis*, *B. canis*, *B. melitensis*, and *B. ovis*. Based on the presence or absence of the lipopolysaccharide (LPS) O-antigen, these species are classified as smooth (*B. abortus*, *B. suis* and *B. melitensis*) or rough (*B. canis* and *B. ovis*) (1).

In dogs, brucellosis is primarily caused by *B. canis* (11), which is associated with abortions, neonatal death, epididymitis, infertility, orchitis, prostatitis, lymphadenopathy, splenomegaly, and discospondylitis (7, 9, 11, 22, 24). However, canine infections with other *Brucella* species, namely *B. abortus* (2, 5, 12, 18, 19, 20, 25) and *B. suis* (8, 14, 15, 23), have also been reported. Infection of dogs with smooth strains is sporadic, and occurs by direct contact with bovine or porcine placenta or discharge from parturitions or abortions (2, 5, 19) as well as by ingestion of contaminated meat from infected animals (23). The aim of this report was to describe the clinical, serological, bacteriological, and pathological findings in a case of brucellosis caused by smooth strain of *Brucella* in a male dog.

Case description

A 6-year-old intact male mixed breed dog, with a body weight of 10 kg, was conducted by activists from an animal welfare non-governmental organization from a rural area near Carcarañá city (Santa Fe Province) to a private

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veterinary clinic. The dog had bilateral perineal hernia associated with dysuria and dyschezia. In addition, small firm testicles with scrotal adhesions were observed. Surgical repair of the hernia and orchiectomy were performed. The presence of antibodies against smooth strains was evaluated in serum using the buffered plate antigen (BPA) and serum agglutination test (SAT) techniques, with 2-mercaptoethanol (2-ME), according to the SENASA guidelines (17). The rapid slide agglutination test (RSAT) was used for the diagnosis of *Brucella canis* (6). The dog tested positive by BPA, SAT, and 2ME, and negative by RSAT for the diagnosis of *Brucella canis*. The case was then referred to the Cátedra de Enfermedades Infecciosas of the Facultad de Ciencias Veterinarias of the Universidad Nacional de Rosario for further analyses. The dog was once again subjected to surgery due to recurrence of the perineal hernia 15 days after the first surgery. On this occasion, samples were taken from the prostate, which was part of the herniated content.

Blood samples were taken at 30 and 45 days after the second surgery for blood culture and serology (BPA, SAT, 2 ME and RSAT). Samples of blood culture were taken using aseptic technique, with butterfly needle from the cephalic antebrachial vein, in a previously shaved area, and placed immediately in blood culture bottles with brain heart infusion broth as base medium (Britania, Argentina). Then they were incubated at 37°C for 30 days in this bottles and aliquots were subcultured weekly in tryptose agar plates (Difco, BD Diagnostic), incubated at 37°C under an atmosphere containing 10% CO2 for 7 days, as previously described (1). The dog sera were positive by BPA and by the complementary tests, with a titer of 1/200 in SAT and1/200 in 2-ME, and negative by RSAT in both samplings. The dog later it died by accident and the studies had to be interrupted.

Prostate samples were at -20°C until subjected to the same culture method used for blood cultures, or fixed in 10% buffered formalin for histopathological and immunochemical analyses.

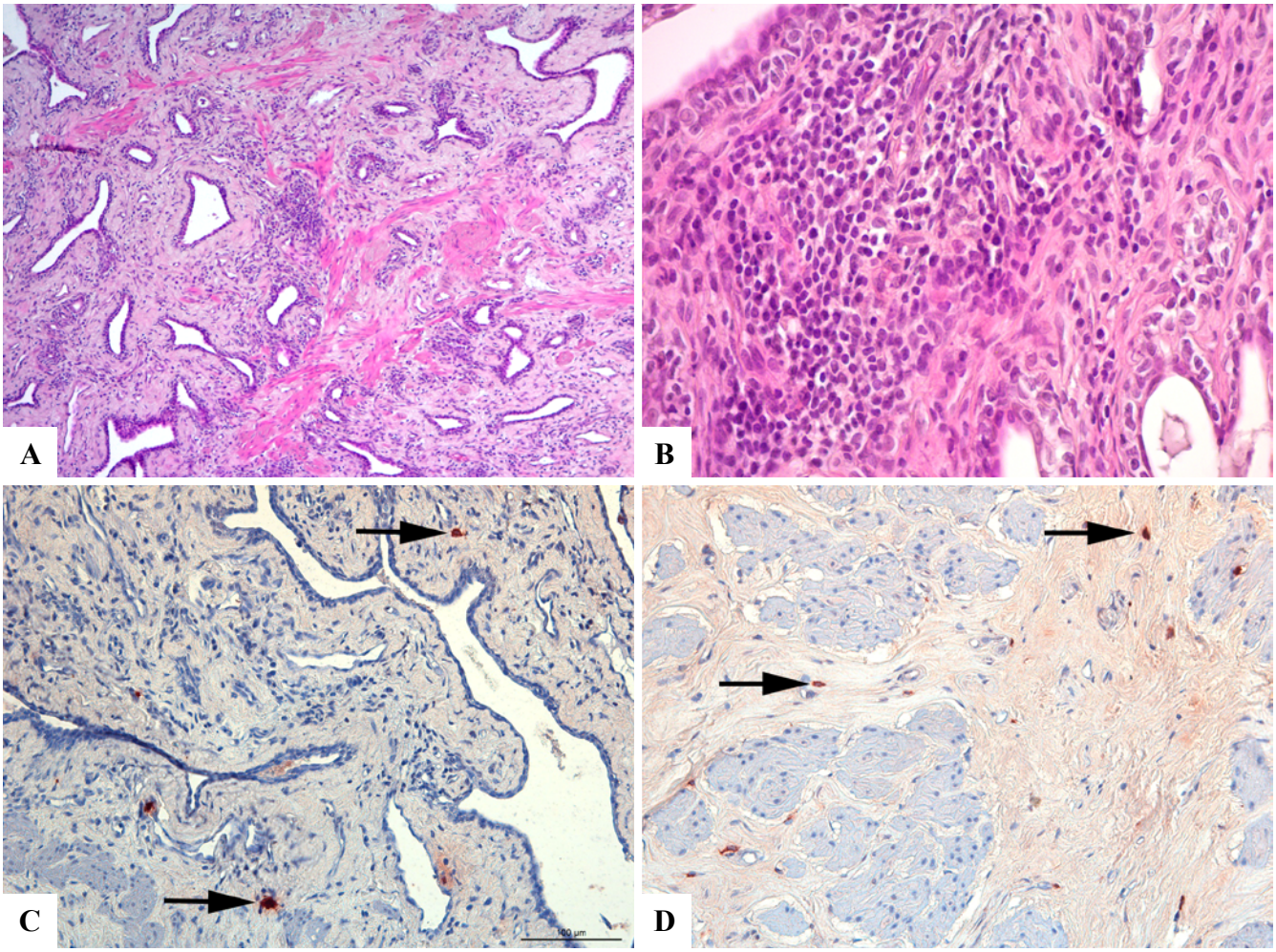

Figure 1. Chronic prostatitis in a domestic dog. **A.** Severe interstitial inflammatory infiltrate and interstitial fibrosis in the prostate associated with glandular atrophy (Hematoxylin and eosin, 5x). **B.** interstitial lymphoplasmacytic and histiocytic inflammatory infiltrate (H&E, 40x). **C.** Prostate, with several brown staining (arrow) in inflammatory cells, mainly associated with macrophages (Immunohistochemistry for detection of *Brucella* spp., 10x). **D.** Prostate, cytoplasmic immunolabelling in macrophages (IHC for detection of *Brucella* spp., 40x).

Formalin-fixed prostate samples were dehydrated, embedded in paraffin, cut (4-µm thick) and stained with hematoxylin-eosin (H&E). In addition, 2 paraffin blocks were submitted to the Universidad de Minas Gerais for detection of *Brucella* spp. by immunohistochemistry as previously described (26). Briefly, the immunohistochemistry technique (IHC) consisted of deparaffinizing and hydrating tissue sections in decreasing grades of alcohol solutions, followed by washing with PBS (NaCl 1.5 M, Na2HPO4 0.1 M, NaH2PO4 0.01 M). Sections were then incubated with 10% hydrogen peroxide for 30 min followed by washing with PBS, incubated with skimmed milk solution (25 µg/mL) for 45 min, followed by PBS washing. Sections were then incubated for 1 h with the rabbit primary polyclonal antibody against *Brucella* spp. (diluted in PBS 1:1000). Sections were washed three times with PBS and incubated with biotinylated secondary antibody for 20 min, followed by washing with PBS and incubation for 20 min with streptavidin peroxidase complex (LSAB+ Kit, DAKO, USA). The reaction was developed with 3, 3'-diaminobenzidine (DAB) (Sigma-Aldrich) and sections were counterstained with Mayer's hematoxylin. Tissues of mice experimentally infected with *B. abortus* were used as positive controls, whereas the negative controls consisted of the same tissues in which the primary antibody was replaced with PBS.

Microscopically, an interstitial lymphoplasmacytic and histiocytic inflammatory infiltrate was observed in the prostate, associated with fibrosis and loss of glandular tissue. In addition, there were areas with fibroplasia, degenerated glandular epithelium, and hyperplasia of glandular ducts and acini (Fig. 1 A-B). Immunostaining was positive, showing brown deposit of granular material in the cytoplasm of some macrophages (Fig. 1 C-D). Results of blood cultures and of prostate tissue culture were negative for the bacterial isolate.

Discussion

In this case, the diagnosis of chronic prostatitis due to brucellosis was based on clinical, serological, pathological and immunohistochemical findings. Serological diagnosis allowed us to attribute prostatitis to a smooth strain of *Brucella*, since the dog serum was positive by BPA and complementary tests (SAT and 2-ME), and negative by the serological test for the detection of *B. canis* (RSAT). It has been recently demonstrated that dogs infected with *B. canis* do not generate antibodies against smooth strains (13). The present report shows the need to apply different complementary techniques to reach a diagnosis of brucellosis in dogs and that the smooth *Brucella* strains can also cause prostatitis, as previously reported (23). Although *B. canis* is the most frequent strain in dogs (24), *B. abortus* and *B. suis* can also cause chronic prostatitis, as observed in infections with *B. canis*. Our case study also indicates the importance of pathological studies in the presence of alterations in the prostate with the aim of characterizing the lesions and differentiate them from other pathologies, such as benign prostatic hyperplasia, neoplasia, and other infectious causes of prostatitis (21).

There is scant information published about pathological findings in cases of dog brucellosis caused by smooth strains (2, 8, 12, 14, 15, 19, 23). In various reports only clinical findings are described, including abortion, epididymitis/orchitis or spondylitis (8, 12, 14, 15, 23). In this case, the dog had signs of epididymitis / orchitis, but this lesion was not microscopically characterized because the testicles were discarded at the time of orchiectomy. Therefore, only the prostate was later available for histopathology. Microscopical lesions observed in the prostate in this case were consistent with previously published reports (4, 11). In this case, immunolabelling of *Brucella* sp. was stronger and observed more frequently in areas of the prostate with moderate to severe inflammatory lesions compared to those areas with milder lesions. This suggests that these lesions, were in fact, due to *Brucella* sp. infection. Ideally, a definitive diagnosis of brucellosis in dogs is based on isolation of the bacterium from tissue samples, discharge, blood, and/or semen. Supportive evidence comes from positive serologic agglutination testing, other serologic titers (7, 21). Difficulty lies with the fluctuant level and length of the bacteremia in the dog. Immunohistochemistry has previously been used for the diagnosis of *Brucella canis* (22) and *Brucella abortus* (26). Indeed, immunohistochemistry is an excellent alternative to bacterial culture as a method of diagnosis in cases of brucellosis.

Unfortunately, the strain was not isolated and, therefore, it was not possible to futher characterize the *Brucella* species in this case. The fact that it was not isolated from the prostate may be due to storage temperature (-20°C). Scalan et al. (20) mentioned that freezing of tissues reduces *Brucella* viability. Nevertheless, immunochemical analysis showed several macrophages with intracytoplasmic immunelabelling, demonstrating that the *Brucella* strain was established in that organ. According to the literature, bacteremia by *B. canis* in dogs often recurs, with the prostate being the colonization organ, among others. Smooth strains of *Brucella* produce a short bacteremia and then localize in reproductive organs and in lymphoid organs (9, 20). The fact that it was not isolated in the two blood cultures may be due to the absence or very low presence of bacteria in the blood stream at the moment of sampling.

Argentina is an endemic country for brucellosis caused by *B. melitensis*, *B. abortus*, *B. suis* and *B. canis*, being a very important zoonosis in the region (10). Brucellosis is a common cause of abortion in beef and dairy cattle (16). Information about the seroprevalence of smooth *Brucella* strains in dogs in Argentina is scarce. A serological study focusing on the circulation of smooth strains in canines from General Pico (La Pampa) showed a prevalence of smooth strains close to 3% (3). There are few reports in the scientific literature about clinical cases of canine brucellosis

caused by smooth strains in Argentina. The only reported case consists of abortion in a dog due to *B. suis* biotype 1 infection in the locality of Rio Cuarto (Córdoba) (14). There are records in Australia (15) and the Netherlands (23) associated with *B. suis*, and cases of *B. abortus* reported in Brazil (12). One of the cases of canine infection by *B. suis* in The Netherlands (23) was due to the consumption of *B. suis*-infected meat imported from Argentina. We believe that this disease is underdiagnosed in dogs in Argentina, possibly because of the lack of knowledge among professionals about the possibility that these strains may affect dogs. Other reasons include the lower frequency of veterinary care of dogs from rural areas, and the fact that this infection cannot be diagnosed in all cases (4). These findings show the need to perform seroprevalence studies of smooth strains in canines from rural localities, taking into account that farm animals are the main hosts of these pathogens.

We conclude that this dog was affected by smooth strains, generating pathological alterations difficult to distinguish from those caused by *B. canis*, and that routine serological tests should always be performed, regardless of the origin of the dog (rural, semi-urban or urban) to discard all the *Brucella* species.

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